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The association between neutrophil lymphocyte ratio, vitamin (D) deficiency and development of peripheral neuropathy in type 2 diabetic patients

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ABSTRACT

Background/Aims: Some studies had suggested that neutrophil lymphocyte ratio (NLR) levels grow with the increase of nerve conduction velocity (NCV). Recently, several studies found that vitamin (D) deficiency can cause the risk of diabetes mellitus (DM) and its complication including neuropathy.The aim of our study is to evaluate the association betweenNLR, vitamin (D) deficiency and development of peripheral neuropathy in type 2 diabetic (T2DM) patients.

Methods: In cross sectional study we selected a total of 60 T2DM patients.All patients underwent nerve conduction study (NCS). Then we divided them according to the results into two groups, 45 patients with DPN and 15 patients without DPN as a control group.DPN group wassubdivided according to NCS results into three groups,11 patients with mononeuropathy,4 patients with mononeuropathy multiplex and30 patients with polyneuropathy. We evaluated the effect of DPN in these patients as regarded NCS results on HbA1c levels, NLR and vitamin (D) levels.

Results: There were very highly statistically significant differences between DPN group and non-DPN group as regard HbA1c and [25(OH) D] levels, ($P < 0.001$*** for all). There was no statistically significant difference between them as regarded NLR, ($P = 0.501$). Multivariate logistic regression analysis showed that, [25(OH) D] levels and duration of DM were independent risk factors for DPN, ($P = 0.041$*, OR = 0.88, 95% CI = 0.78-0.10), ($P = 0.016$*, OR = 1.54, 95% CI = 1.09-2.18) respectively.

Conclusions: Vitamin (D) deficiency and HbA1c levels may be independent risk factors for development of DPN in T2DM in Minia. It is important to supply vitamin (D) appropriately and control of diabetes for preventing the generation of DPN in T2DM. NLR can't be a predictor for DPN.

Abbreviations: NLR = Neutrophil lymphocyte ratio**, NCS =** nerve conduction study, **NCV** = nerve conduction velocity, **DPN** = diabetic peripheral neuropathy**, DM** = diabetes mellitus and **T2DM** = type 2 diabetes mellitus**.**

Keywords: Neutrophil lymphocyte ratio**,**vitamin D**,** nerve conduction study, nerve conduction velocity, diabetic peripheral neuropathy andtype 2 diabetes mellitus.

INTRODUCTION

Type 2 diabetes (T2DM) is a major public health problem worldwide(1). The number of people with diabetes will rise up to 366 million in 2030 which is estimated by the World Health Organization (WHO) (2). Diabetic peripheral neuropathy (DPN) is a major public health problem worldwide (1). It is one of the main causes of morbidity and increased mortality (2). Studies of American Diabetes Association (ADA) showed that 26.4% of T2DM patients are complicated by painful DPN, while up to 50% of the DPN patients may be asymptomatic(3).

Diagnosing DPN depends on clinical examination and nerve conduction study (3). Further development of DPN can lead to diabetic foot lesions as ulcers, infection, gangrene of the feet and amputation (4). These late complications are associated with a higher mortality and reduced quality of life and impose a great cost on healthcare systems (5). Therefore, searching for a reliable indicator for the early diagnosis of DPN is of most significance.

Epidemiological studies have indicated the main risk factors of DPN including the high level of blood glucose and glycated hemoglobin(6). Neutrophil lymphocyte ratio (NLR) levels grow with the increase of nerve conduction velocity (NCV) (1). Recently, increasing studies have been carried out to explore the association between vitamin (D) level and the development of DPN in patients with diabetes mellitus (DM). They found that, vitamin (D) deficiency can cause the risk of DM and its complication including neuropathy (2).

Therefore, we performed this cross sectional study to evaluate the association between NLR, vitamin (D) deficiency and development of peripheral neuropathy in T2DM.

MATERIALS AND METHODS

This cross sectional study was conducted in Internal Medicine department and outpatient clinic at Minia University Hospital from November 2018 till November 2019. It included 60 patients with T2DM.

Ethical aspects: All participants were informed about this study before any procedure. Furthermore, this study was performed according to the ethical guidelines of 1975 Declaration of Helsinki after approved by the Institutional Review Board (IRB) for human subject research at faculty of Medicine, Minia University.

Exclusion criteria:In this study weexcluded patients with cardiac problems, renal problems, active infection, acute massive hemorrhage, blood diseases that affect neutrophils and lymphocytes (e.g., myeloproliferative disease and leukemia), cancer, received blood transfusion during the last 2 weeks, received immunosuppressant or steroid drugs during the last 2 weeks or receiving vitamin supplementation.

Medical data:Patients were evaluated by a uniform questionnaire survey asgender, age, smoking status, duration of DM, family history of DM, drug history (receiving oral anti-diabetic drugs or insulin) and other chronic illness.

Anthropometric measures:We measured body weight, height and calculation of body mass index (BMI) as weight divided by height squared (kg/m2) (7).Systolic and diastolic blood pressures were measured in the upper arm by using mercury sphygmomanometer**.**

Nerve conduction study (NCS):NCS was done to all patients. According to their results, the patients were divided into 45 patients with DPN (group I) and 15 patients without DPN (group II) as a control group. Patients with DPN were subdivided into 11 patients with mononeuropathy, 4 patients with mononeuropathy multiplex and 30 patients with polyneuropathy.

In our study, DPN was defined as the presence of one or more abnormal nerve conduction results (amplitude or conduction velocity) (8)in the form of mononeuropathy, mononeuropathy multiplex or polyneuropathy in T2DM patients. Normal NCS; is Normal findings on all tests. Mononeuropathy**;** is a lesion restricted to one single peripheral nerve, producing the characteristic motor, sensory and reflex abnormalities distal to the site of the lesion. Mononeuropathy multiplex**;** is the term used to describe the coexistence of two or more separate mononeuropathies in one limb and this most usually occur in diabetes mellitus and vasculitis. Polyneuropathy;is a generalized neuropathy affecting all peripheral nerve fibers (9).

Laboratory investigation:Participants' laboratory tests were taken from hospital records as fasting plasma glucose (FPG), 2 hours post prandial blood sugar (2hpp), glycated hemoglobin (HbA1c), complete blood picture (CBC), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), lipid profile, urea, renal and liver function tests, hepatitis C virus antibodies (HCV Ab), [25(OH) D] levels and uric acid. All these investigations were measured to exclude other causes of DPN and to confirm our study.

Statistical analysis:The analysis of the data was carried out using the IBM SPSS 20.0 statistical package software. Data were expressed as mean ± SD, minimum and maximum of range for quantitative parametric measures in addition to both number and percentage for categorized data.

Student t-test and analysis of variance (ANOVA) were used for comparison between independent groups for parametric data and the Chi-square test or Fisher's exact testwere used to compare categorical variables.

Pearson's and spearman correlation analysis was used to describe the association between numerical variables. Binary logistic regression analysis was conducted to calculate adjusted odds ratios. A *P*-value of 0.05 or less was considered significant.

RESULTS

Our study included 60 T2DM patients.NCS was done to all of them, which divided them into two groups, 45 patients with DPN (group I) and 15 patientswithout DPN (group II) as a control group. Patients with DPN were subdivided into 11 patients with mononeuropathy, 4 patients with mononeuropathy multiplex and 30 patients with polyneuropathy.

A) Analysis of data between our main two groups:

- **Demographic data:**

  This study included 16 (26.7%) males and 44 (73.3%) females. The mean age was (50.11±10) years for group I, ranged from 30 to 75 and (48.40±12) years for group II, ranged from 32 to 72. When we compared our groups, we found that, there was a statistical significant difference between them as regarded duration of DM (P = 0.030*).

- **Anthropometric measures:**

  There were no statistically significant differences between them as regard weight, height, body mass index (BMI), systolic blood pressure (SBP) and diastolic blood pressure (DBP), (P = 0.081, 0.385, 0.162, 1.000 and 0.972 respectively).

- **Laboratory data:**

  As shown in table [1, 2], there were very highly statistically significant differences between them as regarded HbA1c, microalbuminuria, [25 (OH) D] levels and [25 (OH) D] interpretations, (P < 0.001*** for all). There were statistically significant differences between them as regard 2 hours

postprandial blood sugar (2hpp) and uric acid (P = 0.035* and 0.049* respectively). There were no statistically significant differences between them as regarded NLR, HCV Ab levels, LDL, HDL, triglyceride and total cholesterol (TC) (P= 0.501, 0.427, 0.895, 0.312, 0.922 and 0.801 respectively).

Table [1]: Comparison between our two groups:

	Group I (Range) Mean ± SD	Group II (Range) Mean ± SD	P value
FPG (mg/dl)	(79-311) 160.29±56.72	(78-220) 148.67±35.41	0.460
2hpp (mg/dl)	(95-500) 278.76±88.19	(137-308) 224.73±69.4	**0.035***
HbA1c (%)	(4.4±11.1) 8.14±1.71	(5.1±8.6) 6.55±0.94	**0.001*****
Neutrophils (%)	(27-65) 50.56±9.11	(35-70) 51.47±9.82	0.743
Lymphocytes (%)	(20-64) 39.16±9.58	(17-53) 37.2±9.5	0.495
NLR	(0.42-3.25) 1.43±0.62	(0.67-4.12) 1.57±0.84	0.501
[25 (OH) D] levels (ng/ml)	(4.6±35) 13.74±8.95	(8.5±36) 27.97±7.75	**<0.001*****
Microalbuminuria (mg)	(6-49) 25.09±13.87	(2.1-5.8) 3.72±0.83	**<0.001*****
Uric acid	(3.6-8.3) 5.99±1.03	(3.2-7.7) 5.37±1.03	**0.049***
LDL (mg/dl)	(45-161) 100.91±26.29	(54-168) 99.73±38.73	0.895
HDL (mg/dl)	(25-71) 38.51±10.74	(23-60) 35.33±9.5	0.312
TGs (mg/dl)	(41-323) 143.89±82.06	(51-272) 146.27±76.72	0.922
TC (mg/dl)	(85-274) 168.8±42.37	(96-252) 165.53±45.95	0.801

- SD: standard deviation, FPG: fasting plasma glucose, 2hpp: 2 hours post prandial blood sugar, NLR: neutrophil lymphocyte ratio, [25 (OH) D]: 25 hydroxyvitamin (D), LDL: low density lipoprotein, HDL:high density lipoprotein, TGs: triglyceride, TC: total cholesterol.
- * Significant association at P value < 0.05, ** highly significant difference at P value < 0.01, *** very highly significant difference at P value < 0.001.

Table [2]: comparison between our two groups as regarded HCV Ab and vitamin (D) interpretations:

		Group I (45)	Group II (15)	P value
[25 (OH) D] interpretation	- Deficient - Insufficient - Sufficient	23 (51.1%) 17 (37.8%) 5 (11.1%)	1 (6.7%) 3 (20%) 11 (73.3%)	**<0.001*****

		Group I (45)	Group II (15)	P value
HCV Ab	+ve -ve	8 (17.8%) 37 (82.2%)	1 (6.7%) 14 (93.3%)	0.427

- SD: standard deviation, ,[25 (OH) D]: 25 hydroxyvitamin (D), HCV Ab: hepatitis C virus antibodies.
- * Significant difference at P value < 0.05, ** highly significant difference at P value < 0.01, *** very highly significant difference at P value < 0.001.

B) Analysis of data between our subdivision groups:

➢ **Demographic data:**

There was a statistically significant difference between our subdivision groupsas regarded duration of DM (P =0.023*).

➢ **Anthropometric measures:**

There were statistically significant differences between our subdivision groups as regard height, BMI (p = 0.029* and 0.016* respectively).

➢ **Laboratory data:**

As shown in table [3, 4], there were very highly statistically significant differences between our subdivision groups as regard microalbuminuria and [25(OH) D] interpretation, (P < 0.001*** for both). There was highly statistically significant difference between them as regard HbA1c, (P =0.005**). There were no statistically significant differences between them as regard [25(OH) D] and HCV Ab levels, (P = 0.093 and 1.000 respectively).

Table [3]: Comparison betweenour subdivision groups:

	Mononeuropathy group	Mononeuropathy multiplex group	Polyneuropathy group	P value
	Mean ± SD	Mean ± SD	Mean ± SD	
FPG (mg/dl)	149.3±42.5	157±47	164.8±62.9	0.744
2hpp(mg/dl)	254.91±78.29	266±98.33	289.20±91.30	0.530
HbA1c (%)	6.80±1.41	7.85±1.69	8.67±1.57	**0.005****
Neutrophils (%)	50.55±9.67	45.25±13.87	51.27±8.33	0.473
Lymphocytes (%)	40.45±10.14	45.25±14.73	37.87±8.59	0.313
NLR	1.38±.59	1.15±.63	1.49±0.63	0.575
[25 (OH) D] (ng/dl)	18.82±2.48	11.50±1.91	12.18±10.30	0.093
Microalbuminuria (mg)	7.14±0.48	9.35±.72	33.77±7.59	**<0.001 *** **
Uric Acid (mg/dl)	5.30±1.13	5.35±.26	5.56±0.93	0.776
LDL (mg/dl)	103±17.96	108.00±16.79	99.20±29.95	0.791
HDL (mg/dl)	40.09±10.46	38.75±10.56	37.90±11.16	0.851
TGs (mg/dl)	158.55±91.09	144.00±118.71	138.50±76.07	0.794
TC (mg/dl)	178.27±30.45	189.25±55.72	162.60±44.18	0.354

- SD: standard deviation, FPG: fasting plasma glucose, 2hpp: 2 hours post prandial, NLR: neutrophil lymphocyte ratio, [25 (OH) D]: 25 hydroxyvitamin (D), LDL: low density lipoprotein, HDL:high density lipoprotein, TGs: triglyceride, TC: total cholesterol.

- * Significant association at P value < 0.05, ** highly significant difference at P value < 0.01, *** very highly significant difference at P value < 0.001.

Table [4]: Comparison betweenour subdivision groups as regarded HCV Ab and vitamin (D) interpretation:

		Mononeuro-pathy group (11)	Mononeuro-pathy multiplex group (4)	Polyneuro-pathy group (30)	P value
[25 (OH) D] interpretation	Deficient Insufficient Sufficient	0 (0%) 11 (100%) 0 (0%)	0 (0%) 4 (100%) 0 (0%)	23 (76.7%) 2 (6.7%) 5 (16.6%)	**<0.001*** **
HCV Ab	+ve -ve	2 (18.2%) 9 (81.8%)	0 (0%) 4 (100%)	6 (20%) 24 (80%)	1.000

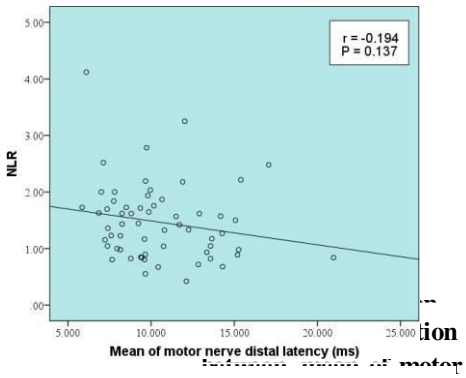
- SD: standard deviation, [25 (OH) D]: 25 hydroxyvitamin (D), HCV Ab: hepatitis C virus antibodies.
- * Significant association at P value < 0.05, ** highly significant difference at P value < 0.01, *** very highly significant difference at P value < 0.001.

C) Correlation between mean of nerve distal latency (DL), amplitude, NCV and different variables in our cases:

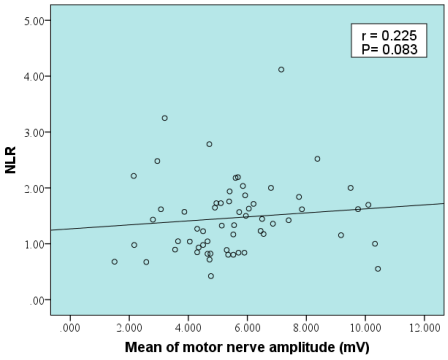
➢ **Motor**:

The mean of motor nerve distal latency (DL) was calculated using the formula: motor nerve DL = DL of right and left motor nerves of (median nerve + ulnar nerve + posterior tibial nerve + common peroneal nerve)/8. The mean of motor nerve amplitude and motor NCV were calculated respectively using the same method. Spearman's correlation analysis was done between:

- Mean of motor nerve DL and different variables, which revealed that, there were negative correlation between it and NLR (r = -0.194, P = 0.137) and [25(OH) D] levels (r = -0.271, P = 0.036*), as shown in figure [1, 4].
- Mean of motor nerve amplitude and different variables, which revealed that, there were positive correlation between it and NLR (r = 0.225, P = 0.083) and [25(OH) D] levels (r = 0.159, P = 0.226), as shown in figure [2, 5].
- Mean of motor NCV and different variables, which revealed that, there were positive correlation between it and NLR (r = 0.061, P = 0.645) and [25(OH) D] levels (r = 0.304, P = 0.018*), as shown in figure [3, 6].



Figure[1]:Correlationbetweenmean of motor nerve DL andNLR.



Figure[2]:Correlationbetweenmean of motor nerve amplitudeand NLR.

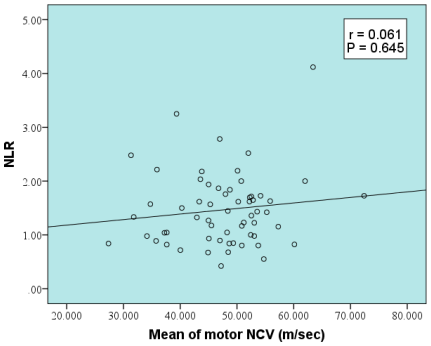
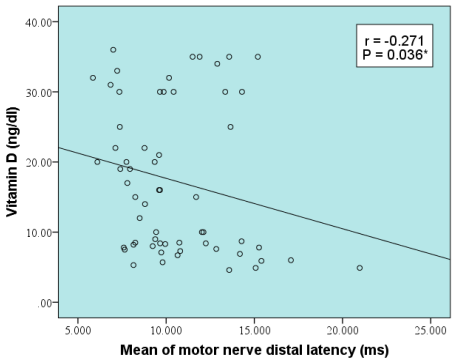


Figure [3]: Correlation between mean of motor NCV and NLR.



Figure[4]:Correlationbetweenmean of motor nerve DL andvitamin(D).

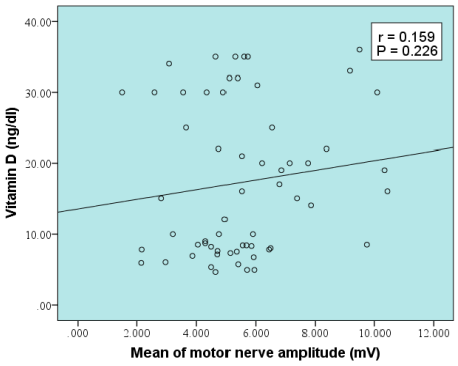
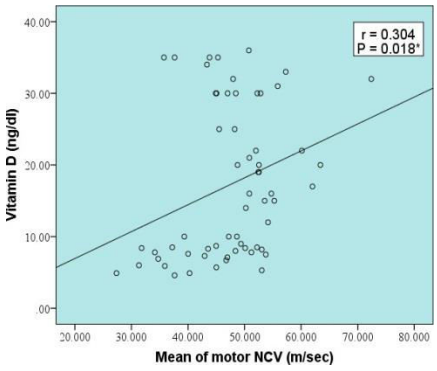


Figure [5]: Correlation betweenmean of motor nerve amplitudeand vitamin(D).



Figure[6]:Correlationbetweenmean of motor NCV andvitamin(D).

- **Sensory:**

The mean of sensory nerve distal latency (DL) was calculated using the formula: sensory nerve DL = DL of right and left sensory nerves of (median nerve + ulnar nerve + sural nerve)/3. The mean of sensory nerve amplitude and sensory NCV were calculated respectively using the same method. Spearman's correlation analysis was done between:

- Mean of sensory nerve distal latency and different variables, which revealed that, there was positive correlation between it and NLR (r = 0.029, P = 0.828), while there was negative correlation between it and [25(OH) D] levels (r = -0.430, P < 0.001***), as shown in figure [7, 10].
- Mean of sensory nerve amplitude and different variables, which revealed that, there were positive correlation between it and NLR (r = 0.106, P = 0.420) and [25(OH) D] levels (r = 0.438, P < 0.001***), as shown in figure [8, 11].
- Mean of sensory nerve conduction velocity and different variables, which revealed that, there was positive correlation between it and [25(OH) D] levels (r = 0.516, P < 0.001***), while there was negative correlation between it and NLR (r = -0.043, P = 0.744), as shown in figure [9, 12].

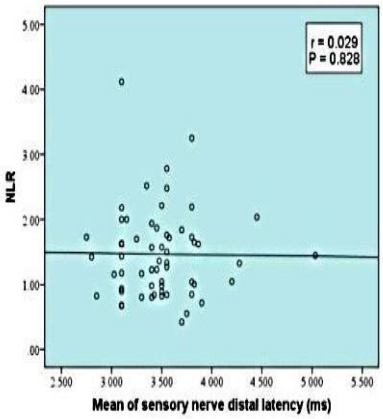


Figure [7]: Correlation between mean of sensory nerve DL and NLR.

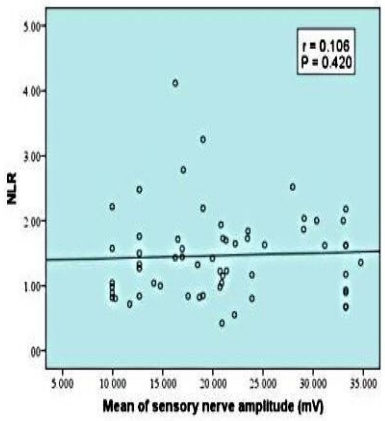


Figure [8]: Correlation between mean of sensory nerve amplitude

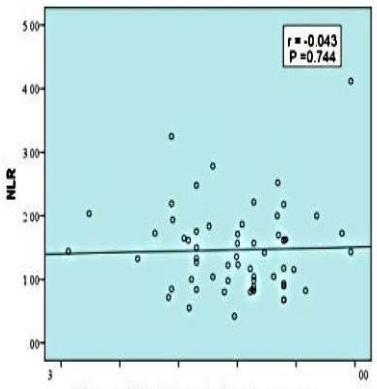


Figure [9]: Correlation between mean of sensory NCV and NLR.

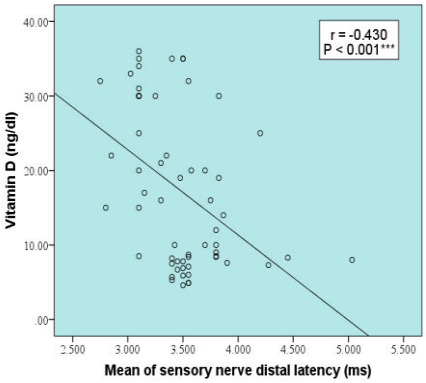


Figure [10]: Correlation between mean of sensory nerve DL and vitamin D.

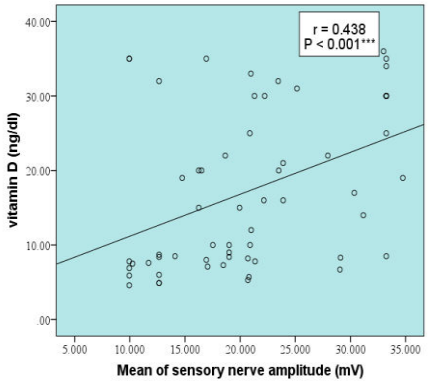


Figure [11]: Correlation between mean of sensory nerve amplitude and vitamin D.

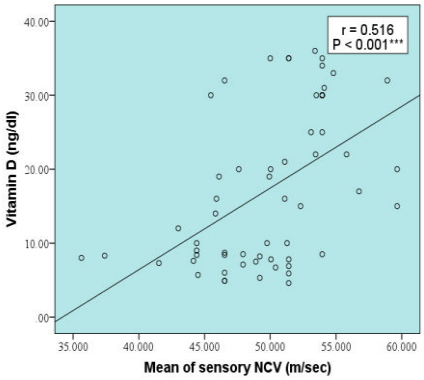


Figure [12]: Correlation between mean of sensory NCV and vitamin D.

D) Binary logistic regression analysis:

Binary logistic regression analysis for prediction of DPN in T2DM patients revealed that, Duration of DM was an independent risk factor for DPN, (P = 0.039*, OR = 1.16, 95% CI = 1.01-1.34). Multivariate logistic regression analysis showed that, Duration of DM was an independent risk factor for DPN, (P = 0.016*, OR = 1.54, 95% CI = 1.09-2.18). [25(OH) D] levels were independent risk factors for DPN, (P < 0.001***, OR = 0.86, 95% CI = 0.79-0.93). Multivariate logistic regression analysis showed that, [25(OH) D] levels were independent risk factors for DPN, (P = 0.041*, OR = 0.88, 95% CI = 0.78-0.10). 2hpp levels were independent risk factors for DPN, (P = 0.042**, OR = 1.01, 95% CI = 1-1.02). HbA1c levels were independent risk factors for DPN, (P = 0.004**, OR = 2.02, 95% CI = 1.254-3.25).

Table [5]: Binary logistic regression analysisfor prediction of DPN in T2DM patients:

	DPN			
	Crude OR (95% CI)	**P value**	**Adjusted OR (95% CI)**	**p value**
Duration of DM	1.16 (1.01-1.34)	**0.039***	1.54 (1.09-2.18)	**0.016***
[25(OH) D] levels (ng/dl)	0.86 (0.79-0.93)	**<0.001*****	0.88 (0.78-0.10)	**0.041***
2hpp (mg/dl)	1.01 (1-1.02)	**0.042***	1.01 (0.99-1.02)	0.466
HbA1c (%)	2.02 (1.254-3.25)	**0.004****	1.32 (0.52-3.35)	0.566

NLR	0.75 (0.32-1.73)	0.497		

- Dependent variable DPN (diabetic peripheral neuropathy), OR (95% CI) (odd ratio and its 95% Confidence Interval), Analysis using binary logistic regression, R^2=0.727.
- DM: diabetes mellitus, [25 (OH) D]: 25 hydroxyvitamin D, 2hpp: 2 hours post prandial blood sugar, HbA1c: hemoglobin A1c, NLR: neutrophil lymphocyte ratio.
- * Significant association at P value < 0.05, ** highly significant difference at P value < 0.01, *** very highly significant difference at P value < 0.001.

DISCUSSION

In our study, when we compared DPN group with non-DPN groupas regarded smoking state, we found that, there was no statistically significant difference between them, (P = 0.627). Our results are against the study of **Xu et al. (2017),** which its multivariate logistic regression analysis showed that, smoking was a risk factor for DPN(3). The meta-analysis by**Clairet al.(2015)**had provided evidence that, smoking was closely linked with the incidence of DPN(10). The discrepancy may be due to limitation in the number of smokers in our study, as participants were mostly female non-smoker.

Also in our study, when we compared DPN group with non-DPN groupas regarded duration of DM, we found that, there was a statistically significant difference between them, (P =0.030*). Multivariate logistic regression analysis showed that, duration of DM was an independent risk factor for DPN, (P = 0.016*, OR = 1.54, 95% CI = 1.09-2.18). Combining the results of multivariate logistic regression analysis and independent sample T test suggest that higher duration of DM may predict a higher incidence of DPN in T2DM. Our results are in agreement with the study of **Su et al. (2018),** which demonstrated that, patients with DPN had a higher diabetic duration than patients without DPN(11).The study of**Lin et al.(2018)** which demonstrated that the duration of T2DM was higher in the DPN group than in the non-DPN group(8).

Also in our study, when we compared DPN group with non-DPN groupas regarded weight, height and body mass index (BMI), we found that, there were no statistically differences between them, (P = 0.081, 0.385 and 0.162 respectively). When we compared our subdivision groups (mononeuropathy, mononeuropathy multiplex and polyneuropathy) as regard height and BMI, we found that, there were statistically significant differences between them, (P = 0.029* and 0.016* respectively). Our results are in agreement with the study of **Li et al. (2015)** which found that BMI was not related to DPN(12).

According to our laboratory data, when we compared DPN group with non-DPN group as regarded fasting plasma glucose (FPG), we found that, there was no statistically significant difference between them, (P = 0.460). Our results are againstthe study of **Garoushi et al. (2019)**, which demonstrated that, FPG was a risk factor for developing DPN(13). The discrepancy may be due to specific group selection by the study of **Garoushi et al. (2019),** as they selected patients who had diabetes for ≥ 5 years, while our study included different patients with T2DM (newly diagnosed and advanced).

Also when we compared DPN group with non-DPN group as regarded 2 hours postprandial blood sugar (2hpp) levels, we found that, there were statistically significant differences between them, (P = 0.035*). Binary logistic regression analysis showed that 2hpp levels were independent risk factors for DPN, (P = 0.042*, OR = 1.01, 95% CI = 1-1.02). Our results are in agreement with multivariate logistic regression analysis of the study of **Xu et al.(2017)**, which showed that, 2hpp levels were risk factors for DPN in patients with diabetes and prediabetes(3).

Also when we compared DPN group with non-DPN group as regarded HbA1c levels, we found that, there were very highly statistically significant differences between them, (P < 0.001***). Binary logistic regression analysis showed that, HbA1c levels were independent risk factors for DPN, (P = 0.004**, OR = 2.02, 95% CI = 1.254-3.25). When we compared our subdivision groups as regard HbA1c

levels, we found that, there were highly statistically significant difference between them, (P = 0.005**). Our results are in agreement with the study of **Yagihashi(2011),** which demonstrated that, the main risk factors of DPN including the high level of blood glucose and glycated hemoglobin(6).

Also when we compared DPN group with non-DPN group as regarded neutrophils, lymphocytes and NLR, we found that, there were no statistically significant differences between them, (P = 0.743, 0.495 and 0.501 respectively). Spearman's correlation analysis between NLR and the mean distal latency/ amplitude/nerve conduction velocity (NCV) levels of motor/sensory nerves showed that, the values of NLR were positively correlated to the mean of motor nerve amplitude (r = 0.225, P = 0.083), motor NCV (r = 0.061, P = 0.645), sensory nerve distal latency (r = 0.029, P = 0.828) and sensory nerve amplitude (r = 0.106, P = 0.420), while they were negatively correlated to the mean of motor nerve distal latency (r = -0.194, P = 0.137) and sensory NCV (r = -0.043, P = 0.744). Binary logistic regression analysis showed that, NLR was not a risk factor for DPN, (P = 0.497, OR = 0.75, 95% CI=0.32–1.73). Combining the results of binary logistic regression analysis, Spearman's correlation analysis and independent sample T test suggest that, we couldn't use NLR as a predictor for DPN in T2DM. When we compared our subdivision groups as regarded NLR, we found that, there was no statistically significant difference between them, (P = 0.575).

Our results are against the study of **Xu et al. (2017),**which found that; neutrophil was positively correlated with DPN while lymphocyte was negatively correlated with DPN. Furthermore, multivariate logistic regression analysis showed that NLR was an independent risk factor for DPN. Combining the results of multivariate logistic regression analysis and independent sample T test suggest that elevated NLR may predict a higher incidence of DPN in T2DM patients(3). The discrepancy may be due to specific group selection by the study of **Xu et al. (2017),** as they selected newly diagnosed T2DM patients, while our study included different patients with T2DM (newly diagnosed and advanced). There were also limitations in the study of **Xu et al. (2017)** in the relationship between the values of NLR and the degrees of severity of DPN, which was not explored. While our study' patients were divided into three groups (mononeuropathy, mononeuropathy multiplex and polyneuropathy) according to their severity of DPN.

Also when we compared DPN group with non-DPN group as regarded [25(OH) D] levels, we found that, there were very highly statistically significant differences between them, (P < 0.001***). Spearman's correlation analysis between [25(OH) D] levels and the mean distal latency/ amplitude/nerve conduction velocity (NCV) levels of motor/sensory nerves showed that, the values of [25(OH) D] were positively correlated to the mean of motor nerve amplitude (r = 0.159, P = 0.226), motor NCV (r = 0.304, P = 0.018), sensory nerve amplitude (r = 0.438, P < 0.001***) and sensory NCV (r = 0.516, P < 0.001***), while they were negatively correlated to the mean of motor nerve distal latency (r = -0.271, P = 0.036) and sensory nerve distal latency (r = -0.430, P < 0.001***). Multivariate logistic regression analysis showed that, [25(OH) D] levels were independent risk factors for DPN, (P= 0.041*, OR = 0.88, 95% CI = 0.78-0.10). Combining the results of multivariate logistic regression analysis, Spearman's correlation analysis and independent sample T test suggest that, lower levels of [25(OH) D] may predict a higher incidence of DPN in T2DM. When we compared our subdivision groups as regarded [25(OH) D] interpretation, we found that, there were very highly statistically significant differences between them, (P < 0.001***).

Our results are in agreement with the meta-analysis of **Qu et al. (2017)**, which demonstrated that, vitamin (D) was involved in the development of DPN, and vitamin D deficiency is very likely to be associated with increased risk of DPN. Appropriate vitamin D supplements can be an effective measurement to prevent the development of DPN in diabetic patients. They found that diabetic patients with vitamin D deficiency are 1.22 times suffering from DPN than those patients without vitamin D

deficiency in Asian. Thus, vitamin D may be a high risk factor for the occurrence of DPN in diabetic patients(2). The study of**Mpandzouet al. (2016)**, which demonstrated that, vitamin D had an immunomodulatory role through its anti-inflammatory and anti-autoimmune actions. Vitamin D deficiency impacted on various central or peripheral neurological diseases(14). The study of **Annweileret al. (2010)**, which demonstrated that, vitamin (D) may act like a neurosteroid hormone during cerebral development in the areas of neurotransmission, neuroprotection and neuroimmunomodulation. Moreover, vitamin (D) deficiency has been associated with neurological and psychiatric disorders. In older adults, hypovitaminosis D has been associated with neuromuscular disorders, dementia, and Parkinson's disease. Thus, vitamin D supplementation might have a protective effect against these neurological disorders(15).

Also when we compared DPN group with non-DPN group as regarded uric acid, we found that, there was a statistically significant difference between them, (P = 0.049*). Our results in agreement with the study of **Lin et al.(2018)**, which showed that, both the amplitude and NCV of sensory/motor nerves were decreased with the increasing serum uric acid (SUA) levels. Furthermore, there were negative correlations between SUA and NCSs (the amplitude and NCV of sensory/motor nerves)(8). The study of **Di Iorio et al.(2006)**,found that, uric acid was significant independent predictors of NCV in the peripheral nervous system during the aging process(16).

Also when we compared DPN group with non-DPN group as regarded microalbuminuria, we found that, there was a very highly statistically significant difference between them, (P < 0.001***). When we compared our subdivision groups as regarded microalbuminuria, we found that, there were very highly statistically significant differences between them, (P < 0.001***). Our results are in agreement with multivariate logistic regression analysis of the study of **Xu et al.(2017)**, which showed that, microalbuminuria levels were risk factor for DPN(3).

Also when we compared DPN group with non-DPN group as regarded HCV antibodies levels, we found that, there were no statistically significant differences between them, (P = 0.427). When we compared our subdivision groups as regard HCV Ab levels, we found that, there were no statistically significant differences between them, (P < 1.000).Our results are against the study of**Aguiar et al. (2019),** which demonstrated that, a high prevalence of cryoglobulinemia and cryoglobulinemicvasculitis were found in Brazilian HCV patients. Cryoglobulinemicvasculitis patients mostly presented non-life-threatening manifestations, especially peripheral neuropathy(17). The discrepancy may be due to limitations in our study, which included only 9 patients with HCV positive, while the study of **Aguiar et al. (2019)** included 68 HCV patients.

Also when we compared DPN group with non-DPN group as regarded LDL, HDL, triglyceride (TG) and total cholesterol (TC), we found that, there were no statistically significant differences between them, (P = 0.895, 0.312, 0.922 and 0.801 respectively). Our results are in agreement with the study of **Franklin et al.(1994)**, which demonstrated that, serum lipid levels were not significantly associated with DPN(18). While our results are against the study of**Lin et al.(2018),** which demonstrated that, the levels of TC were higher in the DPN group than in the Non-DPN group(8). Pearson correlation analysis of the study of **Xu et al.(2017)**, showed that, TG is positively correlated with DPN and multivariate logistic regression analysis showed that triglyceride is risk factors for DPN(3). The study of **Smith and Singleton et al. (2013)** showed that, low HDL, high LDL and cholesterol levels were associated with the development of neuropathy(19). The discrepancy may be due to limitations in the number of our study, as the number of our participants was 60 participants, while the number of them in the study of **Lin et al.(2018)**was 200 participants, the number of them in the study of **Xu et al. (2017)** was 557 participants and the number of them in the study of **Smith and Singleton et al. (2013)** was 218 participants.

Conclusion

We found that vitamin (D) deficiency and HbA1c levels may be independent risk factors for the development of DPN intype 2 diabetes mellitus in Minia. And it is important to supply vitamin (D) appropriately and control of diabetes for preventing the generation of DPN in T2DM. NLR can't be used as a predictor for DPN in T2DM.

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